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THE GERMICIDAL ACTION OF CLEANING AGENTS—A STUDY OF A MODIFICATION OF PRICE'S PROCEDURE

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Much work has been done "in vitro" on the germicidal action of commercial soaps and soaps of the individual fatty acids. Special consideration has been given the germicidal activity of rosin soap by the Naval Stores Research Division[1] in connection with the investigation of the use of rosin and the properties of rosin soaps. To extend these studies to include conditions similar to those of general usage and at the same time to correlate such results with the "in vitro" tests, hand-washing experiments have been considered. Price[2,3] has proposed a procedure for studying the disinfectant action of mechanical cleaners and germicides on the resident flora of the hands. His findings, using this procedure[2,3,4] indicated that commercial bar toilet soaps were inactive as germicides for the resident bacteria of human skin. Since "in vitro" tests have invariably indicated some germicidal activity for such soaps, this was somewhat surprising. Studies were therefore undertaken to determine the validity of Price's findings and the suitability of the procedure suggested by him for experiments using rosin soap.

Using a technic essentially the same as that outlined by Price,[2] practically all of the conclusions drawn by him with regard to rate of removal of the transient and resident flora, effect of soap, influence of vigor of washing, persistency of size and kind of "resident" flora, germicidal action of 70% alcohol, and the inactivity of petroleum ether have been verified. Price showed that the transient flora are easily removed and may vary considerably from time to time while the resident flora remain practically constant and are difficult to remove.

A group of 32 individuals was studied with the object of selecting suitable subjects for subsequent use in testing the germicidal action of rosin soaps on the resident flora of the hands. The total number of organisms washed from the hands of different people and the rate of removal varied tremendously, yet the constancy of the population and the rate of removal for any given individual from week to week was in most cases remarkable. In these studies a period of at least 7 days was allowed between each washing experiment since Price[2] showed that it may require 7 to 14 days for the resident flora to return to normal after washing as prescribed. Individuals were found who removed, by washing, larger numbers of organisms than those reported by Price.[2] The bacterial counts with eight of the individuals varied in such a manner or were so small that their use in the evaluation of the germicidal action of soaps was impossible. The individuals that had abnormally low counts per basin were chemists engaged in work that exposed their hands to such solvents as alcohol, turpentine, acetone, or strong aldehydes.

As the group of individuals examined in these studies were volunteers and included office personnel and sub-professional employees as well as professional employees, observations were not restricted to any one class or type of individuals. Because of the varied character of the groups studied, it was not possible to control the physiological area washed or the vigor of washing as rigidly as in surgery where all individuals are thoroughly familiar with the tedium of hand-washing. In view of this and also to make the conditions of washing more like those of normal practice, it seemed desirable to slightly modify Price's procedure.[2]

A series of 10 enameled basins, each of approximately 7 liters capacity, was used. Before making a series of tests, the basins were washed with soap and water and thoroughly rinsed with hot tap water. Two liters of distilled water at 23 to 28 C. were added to each basin. A quantity of commercial granulated soap equivalent to 2 gm. of anhydrous soap and containing no rosin soap was used for washing the hands at each basin. The hands were moistened and the soap worked into a lather in 20 to 30 seconds, and then the lather was thoroughly worked on the hands for 75 seconds, adding water to maintain a good lather. The last 15 or 20 seconds of the 2 minute period allotted for washing in each basin were spent in rinsing the lather from the hands. No brush was used. At the end of the 2 minute period the process was repeated immediately in the next basin in the series without drying the hands. This made the total washing time 20 minutes. Each individual was allowed to establish the area of the forearms that he desired to wash, in addition to the hands, and then instructed to use as much care as possible in maintaining this area constant. The procedure described above will be called normal when granular soap is used at all basins.

The wash water in each basin was stirred with a sterile pipette and at least two 1 ml. aliquots were transferred to individual sterile petri dishes. Approximately 15 ml. of plain nutrient agar made up with 1.5% agar, 1% peptone, 0.3% beef-extract, and 1% NaCl, and adjusted to p_H 6.9 was added to each plate at a temperature of 45 C. The agar was allowed to solidify and the plates were then incubated at 30 C. for 48 hours. All counts were made using a Quebec colony counting chamber. Control cultures taken from basins containing only water and soap after standing for 60 minutes gave counts varying from 0 to 6 colonies per plate and averaged less than 2 bacteria per ml. The bacterial count of each basin was obtained by multiplying the number of colonies per ml. by 2,000, the number of mls. used in each basin. The average control count of 4,000 per basin was regarded as insignificant as the data will show.

By following the washing procedure outlined above, the removal curves obtained by plotting bacterial count per basin in the order used approaches a straight line as the washing proceeds and, although the curves tend to approach zero, it would seem that an infinite number of washings would be necessary to arrive at that point. The number of bacteria removed from the hands is undoubtedly a function of the number of bacteria on the hands. No attempt was made to establish the original microbial population on the hands prior to washing through calculation or extrapolation of the curves for the cumulative totals of the counts for the 10 basins. Any value for the original population arrived at by extrapolation of a cumulative total curve derived from such counts would depend primarily upon the number of washes in

the series and would have purely a relative value.

The counts obtained with 6 different individuals using granular soap at each basin are given in table 1. They are illustrated graphically in figure 1.

TABLE 1.—*Variations Found between 6 Individuals as Shown by the Number of Bacteria Washed from Hands in Successive Basins*

Number of Individual	Count per Basin, in Thousands									
	1	2	3	4	5	6	7	8	9	10
1	10,628	8,543	6,636	6,272	4,992	4,352	3,542	2,848	2,656	2,496
2	8,256	6,784	5,120	4,158	3,584	3,062	2,816	2,560	2,112	1,984
3	6,010	4,366	3,328	2,240	1,956	1,856	1,664	1,408	1,372	1,309
4	5,184	3,648	2,244	1,360	1,152	1,000	858	812	744	722
5	4,324	2,048	1,920	1,472	1,280	1,172	1,269	1,072	752	634
6	3,024	2,359	1,942	1,390	1,148	968	822	769	725	686

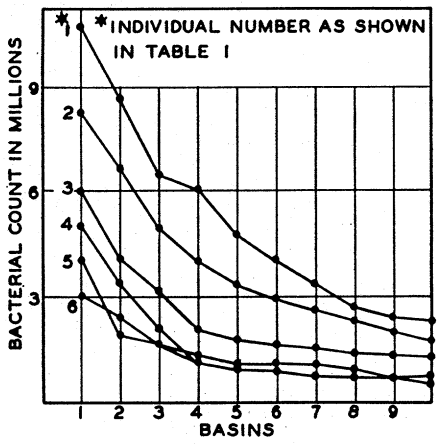

FIGURE 1

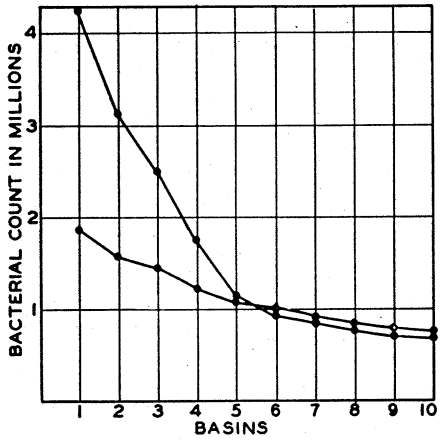

FIGURE 2

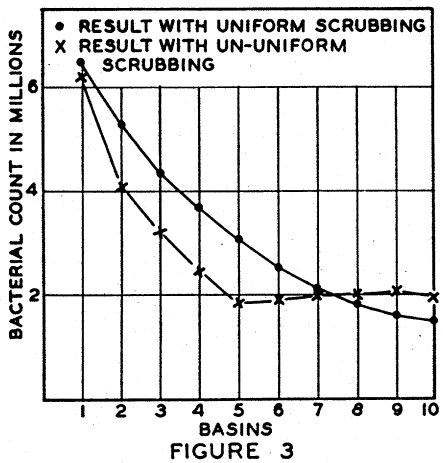

FIGURE 3

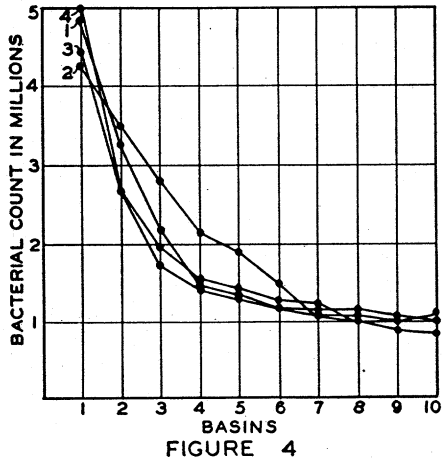

FIGURE 4

Fig. 1.—Removal curves for 6 subjects, plotted from data given in table 1.

Fig. 2.—Most frequent type of deviation observed in the removal curves of the individual.

Fig. 3.—Effect on removal curves of manner of washing.

Fig. 4.—Removal curves for one individual determined at intervals during a 9 month period.

The results in table 1 and figure 1 show that the removal curves for different individuals vary tremendously. Individual 1 repeatedly washed off more than 10 million viable organisms in the first basin, whereas individual 6 never washed off more than 4 million. From figure 1 it may be seen that the rate of removal tended to become less as the initial count became less.

With those individuals considered consistent throughout the duration of the studies, some variations were encountered in the bacterial count per basin from duplicate tests. These variations became smaller as the subject washed in successive basins until after the fifth basin the counts usually were the same as or within the limits of, experimental error. This can be explained, as pointed out by Price[2] on the basis of the variation in the "transient" part of the skin population which are removed by the first washings while the rate of removal of the "resident" flora remains practically constant. An example of this type of variation is shown in figure 2.

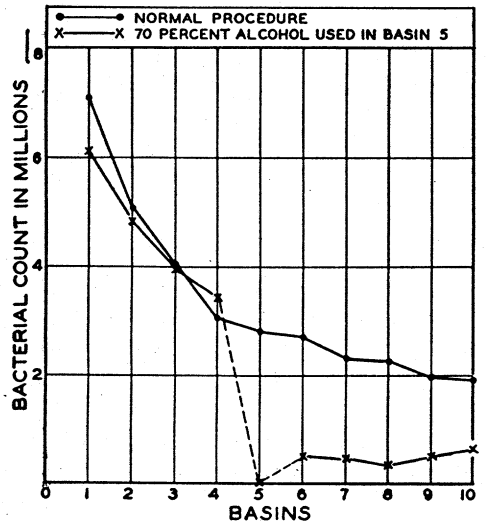

Fig. 5.

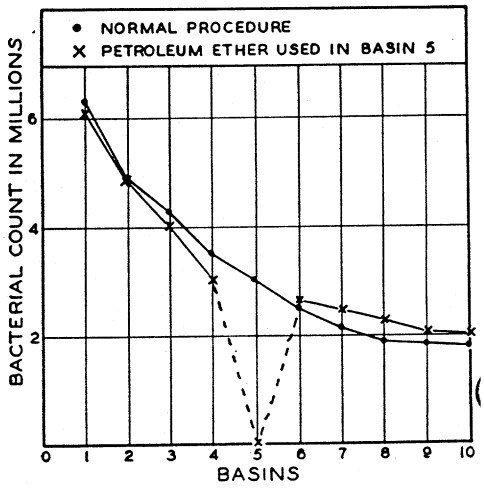

Fig. 6.

Fig. 5.—Effect of washing in basin No. 5 with 17% alcohol.
Fig. 6.—Effect of washing in petroleum ether for 2 minutes in basin No. 5.

It is evident that deviations of the type shown in figure 2 would not influence results from studies on the effect of germicidal materials on the "resident" flora of the hands because the "resident" flora was practically the same at the time of both tests.

A lack of uniformity in washing affects the removal curve. In figure 3, the normal curve for one of the individuals who washed in a uniform manner is compared with the normal curve for a volunteer who had a characteristic fault of not washing uniformly, either with respect to vigor of washing or area washed, and consequently could not be used.

With certain individuals the uniformity of successive removal curves, using the normal procedure at intervals during a nine-month period, was remarkable. In figure 4 are shown 4 curves taken from tests with 1 individual at intervals over a 9 month period.

The results shown in figure 4 are from 1 of the more consistent individuals. However, by averaging the results from 3 or 4 individuals, equally or more uni-

form curves were obtained at any given time. In table 2 the counts of 2 groups of 3 individuals at different times are given. It may be seen that where the counts for the individuals in both groups vary between tests, the averages for 3 individuals are less variable. The first group of 3 individuals, nos. 7, 8 and 9, shown in table 2, are presented as representative of an almost perfect correlation in the averaged values. The second group of 3 individuals, numbers 10, 11 and 12, represent about the maximum variation encountered in a group.

TABLE 2.—*Counts on Individuals and Averages for 2 Groups of 3 Individuals at Different Times*

Group	Interval	Indiv. No.	Count per Basin, in Thousands									
			1	2	3	4	5	6	7	8	9	10
I	1	7	4,932	3,944	2,176	2,176	1,865	1,600	1,536	1,346	1,328	1,160
		8	5,184	3,968	3,200	2,880	2,816	2,368	1,920	1,792	1,472	1,408
		9	8,107	6,336	4,587	2,880	2,268	1,963	1,899	1,877	1,856	1,728
		Average	6,074	4,749	3,321	2,645	2,316	1,977	1,785	1,672	1,552	1,432
	2	7	3,705	3,520	3,456	3,264	2,560	2,240	2,048	1,806	1,664	1,472
		8	7,912	5,056	4,032	3,136	3,072	1,792	1,600	1,536	1,216	1,152
		9	6,783	4,864	3,136	3,008	2,240	2,048	1,984	1,920	1,664	1,536
		Average	6,133	4,480	3,541	3,136	2,624	2,023	1,877	1,754	1,515	1,387
II	1	10	6,010	4,366	3,328	2,240	1,956	1,856	1,664	1,408	1,372	1,409
		11	4,151	2,407	2,343	1,810	1,664	1,346	1,280	1,152	1,152	1,152
		12	4,352	3,198	2,432	1,668	1,124	952	768	665	606	507
		Average	4,838	3,324	2,701	1,906	1,581	1,385	1,237	1,075	1,043	1,023
	2	10	11,520	4,846	3,897	2,955	2,831	2,560	2,624	2,432	2,325	2,423
		11	3,200	2,688	2,242	1,856	1,728	1,600	1,211	1,156	1,024	896
		12	1,796	1,520	1,452	1,172	1,152	984	876	872	843	864
		Average	5,505	3,018	2,530	1,994	1,904	1,715	1,570	1,487	1,397	1,394

Repeated tests on more than 24 generally consistent individuals have shown that the average curve of 3 individuals, is a much more reliable value than the curve for an average individual. The more individuals employed in computing the average, the more constant the value of the curve becomes. Thus, it would seem that 3 or more selected individuals should be employed in studying the effect of any chemical on the flora of the hands.

Following the procedure described, the effect of a germicide on the resident flora of the hands can be evaluated as suggested by Price.[3,4] This involves washing in the normal manner in the first 3 or 4 basins and then subjecting the hands to the treatment being considered followed by washing the hands in the normal manner in the remaining basins of the series. By comparing the removal curve for the basins following the treatment, with the removal curves for the corresponding basins when the normal procedure is followed at all basins, the effect of the treatment on the resident flora of the hands can be evaluated.

When 70% alcohol was introduced into basin 5, the level of the removal curve for basins 6, 7, 8, 9 and 10 was lowered appreciably (fig. 5). This is in harmony with the observation of Price.[3] When washing in alcohol the subjects were instructed to keep the hands immersed in the alcohol as much as possible while washing the hands and forearms continuously. The bacterial count of the basins for normal procedure and for the alcohol tests are given in table 3 for 4 individuals.

Price[2] stated "the resident bacteria are not attached to the skin by extraneous greases or oils" but in these studies certain individuals were unsuitable for the evaluation of germicidal materials because of abnormally low counts and

these individuals were those whose hands had been exposed to the solvents, alcohol, turpentine, and acetone. From the data at hand one cannot be certain whether this is due to a solvent action, germicidal action, or a combination of both. To test the action of petroleum ether on the flora of the hands, 2 liters were used in the fifth basin in the same manner as was done with 70% alcohol. The results are plotted in figure 6.

These figures indicate that the organisms removed, if any, were killed and that the resident flora was not affected.

TABLE 3.—*The Effect of 70% Alcohol* on the Bacteria on the Hands*

Number of Individual	Count per Basin, in Thousands									
	1	2	3	4	5	6	7	8	9	10
	Normal Procedure									
3	6,010	4,366	3,328	2,240	1,956	1,856	1,664	1,408	1,372	1,309
13	6,784	5,440	4,416	3,904	2,496	2,560	2,112	1,984	1,470	1,408
14	10,500	7,552	6,272	5,632	5,668	5,656	4,672	4,608	4,204	4,188
15	5,120	3,072	2,048	1,344	1,152	1,024	960	896	896	882
Average	7,104	5,108	4,016	3,280	2,818	2,774	2,352	2,224	1,986	1,947
	70% Alcohol Used in Basin 5									
3	5,440	4,736	4,160	3,840	0	100	287	236	256	276
13	4,330	2,880	2,176	1,600	0	712	91	63	78	75
14	8,890	7,044	6,528	5,952	5	1,280	1,344	872	1,600	2,048
15	5,696	4,540	3,048	2,245	0	124	166	85	72	63
Average	6,089	4,800	3,978	3,409	1	554	472	314	502	616

However, no definite conclusions were drawn from these results or the observations on alcohol turpentine, and acetone because the mode of action is obscure and undoubtedly is dependent upon such factors as: (1) the germicidal action of the fat solvents, (2) the miscibility of the solvents with water, (3) the protective action of the moisture film for the bacteria on the hands, and (4) the fat solvent properties of individual solvents.

Only the most general observations were made with regard to the bacterial types found with different individuals. Staphylococcus albus was the predominating organism. One individual invariably shed in all basins throughout a 9 month period, an aerobic spore-forming organism that produced a typical spreading colony unlike any observed with the other individuals. Another shed always an aerobic spore former that produced a peculiar, large, globose subsurface colony. The plates from one individual contained an apparently nonpathogenic fungus found in no other case. Confluent subsurface colonies were characteristic in one instance and small pin-point colonies in another. The characteristic qualitative nature of the flora, exclusive of Staphylococcus albus, with the different individuals was so noticeable that it was the subject of frequent discussion.

By averaging the results from the washing experiments of 3 or more individuals who washed in a uniform manner and who did not come in frequent contact with certain organic solvents, the bacteria removal curves could be duplicated from time to time with remarkable agreement. The effect of a germicide on the resident flora of the hands could be evaluated by using it at or in one or more of basins 4, 5 and 6 of the series, and then comparing the removal curve for the subsequent basins at which the granular soap was used, with the removal curve for the normal procedure. The removal curves for the normal procedure before and after a

period of a week or more, during which a material being studied is used on the hands, can also be used to evaluate the germicidal activity of the material on the transient and resident flora of the hands.

The results obtained with this method confirm previous observations made by Price.[2,3] Data and graphs illustrating the principal types of variables are given in this paper. The method as slightly modified has been adopted for studies in this laboratory to evaluate the germicidal action of rosin soaps.

The only objection to this method for routine quantitative testing of mechanical cleaning and disinfectant action of chemicals on the bacterial flora of normal skin would seem to be the large number of subjects required and the amount of labor involved.

Hand-washing experiments of this type provide an ideal illustration of the persistency of bacteria on the human skin. With those individuals who had no specific training in bacteriology, the appearance of the plates and counts recorded left a profound impression. The method would seem to be particularly adaptable for classroom and laboratory instruction.